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Rate of Divergence of Cellular Sequences Homologous to Segments of Moloney Sarcoma Virus

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The RNA genome of the Moloney isolate of murine sarcoma virus (M-MSV) consists of two parts—a sarcoma-specific region with no homology to known leukemia viral RNAs, and a shared region present also in Moloney murine leukemia virus RNA. Complementary DNA was isolated which was specific for each part of the M-MSV genome. The DNA of a number of mammalian species was examined for the presence of nucleotide sequences homologous with the two M-MSV regions. Both sets of viral sequences had homologous nucleotide sequences present in normal mouse cellular DNA. MSV-specific sequences found in mouse cellular DNA closely matched those nucleotide sequences found in M-MSV as seen by comparisons of thermal denaturation profiles. In all normal mouse cells tested, the cellular set of M-MSV-specific nucleotide sequences was present in DNA as one to a few copies per cell. The rate of base substitution of M-MSV nucleotide sequences was compared with the rate of evolution of both unique sequences and the hemoglobin gene of various species. Conservation of MSV-specific nucleotide sequences among species was similar to that of mouse globin gene(s) and greater than that of average unique cellular sequences. In contrast, cellular nucleotide sequences that are homologous to the M-MSV-murine leukemia virus "common" nucleotide region were present in multiple copies in mouse cells and were less well matched, as seen by reduced melting profiles of the hybrids. The cellular common nucleotide sequences diverged very rapidly during evolution, with a base substitution rate similar to that reported for some primate and avian endogenous virogenes. The observation that two sets of covalently linked viral sequences evolved at very different rates suggests that the origin of M-MSV may be different from endogenous helper viruses and that cellular sequences homologous to MSV-specific nucleotide sequences may be important to survival.

The chromosomal DNA of various mammalian species contains multiple copies of nucleic acids that code for endogenous nontransforming oncornaviruses (3, 6, 17). The origins of transforming oncornaviruses are not well defined; they may arise by recombinational events involving helper viruses and additional viral or cellular genetic information (9, 22). The sarcoma viruses in mammalian cells appear to consist of at least two covalently linked parts. One minor portion is unique to the sarcoma virus genome ("sarc" or sarcoma virus-specific sequences), whereas the major portion ("common") is shared with leukemia helper virus (22, 26). The pattern of evolution based on data obtained from complementary DNA (cDNA) derived from entire endogenous helper viruses suggested that the rate of divergence of viral sequences was very rapid and that entire viral genomes might have been transferred between species (2, 17). It was of interest to determine whether each portion of a sarcoma virus genome has complementary nucleotide sequences in cell DNA of various species and whether each portion evolved at similar rates during adaptive radiation of the species. Such an analysis of the avian sarcoma virus (ASV) genome has been in part described (17, 18, 26). The ASV sequences specific for the transforming ASV have been reported to be absent (18) or present with some mismatching in chicken cell DNA (27). The latter report showed conservation of these sequences among related avian species. Extensive data demonstrated that avian helper leukosis virus information is not well conserved in fowl other than chickens (15, 17, 24, 27, 30).

We were able to prepare DNA complementary to Moloney murine sarcoma virus (M-MSV), and isolated two sets of cDNA, one representing the "MSV specific," and the other the "common" region of the M-MSV genome (13). We were able to show that a majority of MSV-

specific nucleotide sequences were found in normal mouse DNA essentially without mismatching in about one copy per cell genome (12). We now report that MSV-specific nucleotide sequences are found in related rodent species with the proportion and fidelity of sequences predicted by taxonomic criteria. The rate of evolution of MSV-specific sequences was found to be roughly equal to that of mouse hemoglobin sequences. In contrast, the covalently linked "common" subset of M-MSV nucleotide sequences diverged rapidly and was almost already undetectable in a species from the *Mus* genus.

MATERIALS AND METHODS

cDNA's. M-MSV was produced by infecting the 319 S+L-clone of MSV-transformed 3T3FL cells with the IC isolate of Moloney murine leukemia virus M-MuLV (1, 10). The supernatant of the chronically infected cells has maintained a two- to threefold excess of M-MSV over M-MuLV for more than 2 years, as determined by biological titrations and C_rt evaluation (13). Virus-containing supernatant fluid was concentrated by 8% polyethylene glycol precipitation and banded through a 20 to 60% sucrose in phosphate-buffered saline gradient. The banded virus was then used to make cDNA by the endogenous reverse transcriptase reaction in the presence of 100 μg of actinomycin D per ml (ICN) (13). The cDNA made was 4 to 6S in size, hybridized 90% with homologous subunit RNA with a $C_rt_{\frac{1}{2}}$ of 0.05 mol · s/liter, and protected more than 90% of iodinated homologous subunit RNA from RNase T_1 digestion at a 3:1 to 4:1 molar excess (13). These experiments suggested that the cDNA was a representative transcript of the MSV-MuLV viral genomes. The cDNA had a 5 to 10% background binding the hydroxylapatite (HAP), which was shown by salt elution-HAP chromatography to be double-stranded DNA (8).

The isolation of cDNA representative of different portions of the M-MSV genome was described (13). Briefly, total MSV-MuLV cDNA was hybridized to an excess of M-MuLV high-molecular-weight RNA, and the hybridizing cDNA was separated from the nonhybridizing portion by HAP chromatography. The fractions were alkali treated, neutralized, and reconcentrated on HAP. The cDNA's were then hybridized to MSV (feline leukemia virus pseudotype) RNA, and hybridizing fractions were separated from nonhybridizing cDNA as before. After several hybridization cycles, isolated cDNA hybridized specifically to MSV-containing RNAs and not to M-MuLV RNA. The cDNA was called "MSV-specific" cDNA. Similarly, a fraction that hybridized equally to MSV RNA and M-MuLV RNA was obtained. This was called "MSV-MuLV common" cDNA. Each cDNA was of a 4S size; each had high T_m values and low $C_rt_{\frac{1}{2}}$ values (0.02 to 0.05 mol · s/liter) with homologous subunit RNA. To determine what portions of the MSV genome each represented, we hybridized the MSV-specific cDNA and MSV-MuLV common cDNA with iodinated M-MuLV and MSV-MuLV subunit RNAs at low (two- to threefold) molar ratios. MSV-MuLV common cDNA protected 75% of MSV-MuLV RNA and 50% of MuLV RNA from RNase digestion. MSV-specific cDNA protected 20% of MSV-MuLV RNA and 0 to 4% of MuLV RNA from RNase digestion. These studies demonstrated that three-quarters of the MSV genome was represented by MSV-MuLV common cDNA, and one-quarter was represented by MSV-specific cDNA (13). cDNA complementary to mouse globin mRNA was a gift of P. Leder.

Cellular DNA-cDNA hybridizations. Cellular DNA was prepared by a modified version of the procedure of Thomas et al. (29). DNA was prepared from cell packs of cultured cell lines of normal adults of the indicated species. DNA was sheared to 4 to 6S size by sonication. DNA-cDNA hybridizations were carried out in 0.75 M NaCl, 70°C, plus 8 to 9 mg of cellular DNA per ml with 0.5 ng of cDNA/ml (2×10^7 cpm/μg), for a total volume of 80 μl. Ten-microliter aliquots were taken at various times. The aliquots were fractionated on 2-cm HAP (lot no. 14333) water-jacketed columns at 50°C with 0.14 M and 0.3 M phosphate buffer (PB) washes. The material in the 0.3 M PB was the reassociated DNA, whereas the 0.14 M PB wash material was the single-stranded, unreassociated DNA (4). Optical density measurements for monitoring cell DNA reassociation and trichloroacetic acid-precipitable counts per minute for monitoring cDNA hybridization were carried out. C_0t values were calculated as described by Britten and Kohne (5) and corrected to a monovalent cation concentration of 0.18 M Na^+. Hybridizations were carried until plateau values were reached at $\geq$10,000 C_0t.

Thermal denaturation profiles. Thermal denaturation profiles were carried out at $\geq$10,000 C_0t using 2-cm water-jacketed HAP columns. The melting profiles were started at 50°C in 5°C increments with 0.12 M PB washes and included 500 to 1,000 cpm of ^{32}P-labeled MSV-MuLV cDNA hybridized with 65S MSV-MuLV RNA as an internal control. cDNA of ^{32}P-labeled mouse globin served as an internal control on a complete set of reassociations with MSV-specific cDNA or MSV-MuLV common cDNA. The internal standard for cellular DNA · cDNA hybridizations consisted of the cell DNA optical reassociation and the [^{32}P]cDNA globin hybridization. The internal standard for T_m values was [^{32}P]cDNA MSV-MuLV hybridized to MSV-MuLV subunit RNA.

RESULTS

Rate of base substitution of M-MSV-specific sequences in mammalian DNA. Purified cDNA representing only MSV-specific sequences was hybridized with cellular DNA of several strains of mice as well as with DNA of related and unrelated mammals. The percentage of hybridization and T_m values were determined by using, in all cases, cDNA of 4S size with low binding to HAP (0 to 2%) and cellular DNA sheared to similar 4 to 6S size with 85 to 95% maximal self-reassociation under the standardized conditions employed. As presented

in Table 1, the normalized 100% result is with the DNA of the heterologous cat cell infected with, and producing M-MSV. The actual hybridization value was 73% at a C_0t value of $\geq 1.2 \times 10^4$ mol·s/liter. It was clear that normal DNA of several mouse strains reacted 70 to 80% relative to the reassociation with DNA of M-MSV producing cells. The 80 to 81°C T_m was quite high and essentially the same with normal mouse cell DNA as with M-MSV-infected cat cell DNA, indicating a very closely matched copy in normal mouse cells. Two related mouse species had a lower percentage of hybridization (~60% of normalized value) with a lower T_m, by 3 to 7°C. Three strains of rat had proportionally less of M-MSV-specific sequences with a 4°C lower T_m. Other rodents such as hamster, *Proamys*, *Sigmodon*, guinea pig, and squirrel were progressively less related. Human, rabbit, and carnivore DNA, representing different taxonomic orders, contained substantially less homologous DNA. Artiodactyl, avian, and teleost DNA had only minimal, very mismatched, homologous DNA.

TABLE 1. *Presence of Moloney sarc sequences in cellular DNAs*

Species[a]	% Hybridized[b]	T_m[c] (°C)	ΔT_m (°C)
MSV-infected cat cell, producer (P521) clone	100	81	0
BALB/c mouse	79	81	0
C3H mouse	79	80	1
C57B mouse	79	81	0
NIH Swiss mouse	71	81	0
3T3FL line, mouse	71	81	0
IC-3T3FL line, mouse, MuLV producer	71	81	0
Mus caroli	61	78	3
m. casteneus	57	74	7
Sprague-Dawley rat	56	77	4
Oliver-Mendel rat	56	77	4
Fischer rat	56	77	4
Praomys natalensis	47	75	6
Hamster	41	72	9
Sigmodon rat	37	72	9
Human	24	70	11
Cat	26	70	11
Squirrel	23	68	13
Rabbit	21	70	11
Dog	21	68	13
Guinea pig	16	60	21
Salmon	9	60	21
Chicken	6	60	21
Calf	6	60	21
0	0	56	25

[a] Cell DNA obtained from liver homogenates by modified Thomas extraction and sonication to 4*S*-size fragments (29), except in the case of 3T3FL, IC-3T3FL, *M. caroli*, *M. casteneus*, human, cat, and dog, where cell packs of established cell lines were extracted. Salmon testes and calf thymus DNA, which were commercial DNAs (Worthington Biochemicals and Miles Research Products, respectively), were reextracted.

[b] Percentage of renatured at C_0t >12,000 mol·s/liter and normalized for maximal hybridization with a single preparation of MSV-specific cDNA of 73% with P521 cell DNA (cat cell infected with MSV and feline leukemia virus) and 0% background.

[c] The T_m is the temperature at which half of the bound cDNA at 60°C−0.12 M PB is eluted, except in the case of salmon, chicken, and 0 DNA, which had little or no bound cDNA at 60°C−0.12 M PB. In those cases in which a structured component was lacking above 60°C, the T_m was taken as the temperature at which half of the bound cDNA at 50°C−0.12 M PB was eluted. The ΔT_m is the difference in degrees Celsius between the T_m of MSV-infected cell DNA and the T_m of the given DNA hydrid.

Although not shown, in all of the above hybridizations the number of homologous copies of MSV-specific sequences was also examined. It was of interest that the number of copies of MSV-specific sequences in several M-MSV-infected cell lines, both mouse and cat, ranged from 1 to 10 (in preparation). In all of the normal cell DNAs of the various species examined, the $C_0t_{1/2}$ values (2,600 mol·s/liter for BALB/c DNA, 2,900 mol·s/liter for NIH Swiss DNA, 2,000 mol·s/liter for Sprague-Dawley and Fischer DNA and 2,500 mol·s/liter for 3T3FL mouse cell line DNA) indicated that whatever proportion of DNA existed in each species, it was present at only one or a few genes per genome (Fig. 1 and reference 12). Mouse cells infected with a producing M-MuLV did not show any qualitative changes in their MSV-specific sequences. The copy number of MSV-specific sequences was also not amplified in those cells.

Rapid divergence of those nucleotide sequences of M-MSV that are also found in M-MuLV. During purification of cDNA's from M-MSV, the subset of cDNA sequences shared with MuLV was isolated by sequential absorption of M-MuLV (13). These "common" nucleotide sequences were able to protect about 75% of M-MSV iodinated 30 to 35*S* RNA and about 50% of M-MuLV iodinated RNA at saturating ratios (13). When tested with mouse endogenous xenotropic viruses whose information is also known to be in mouse cell DNA, 49 to 24% of common sequences of cDNA hybridized (12, 13). Accordingly, common sequences represent about 50% of the M-MuLV genome and somewhat less of the genome of related murine oncoviruses.

Hybridization of cDNA MSV-MuLV common sequences to various cell DNAs yielded a very different result from the MSV-specific se-

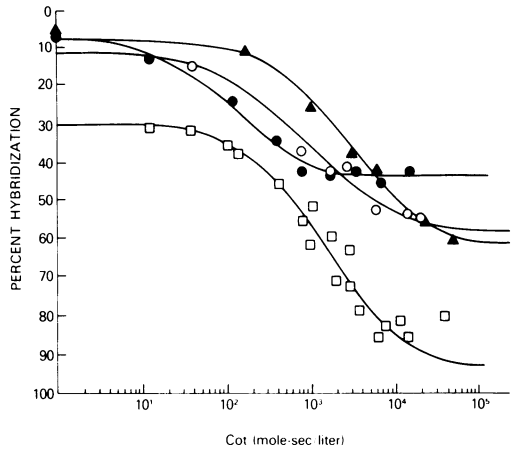

FIG. 1. *Presence and quantity of MSV-specific, MSV-MuLV common, or mouse globin nucleotide sequences in BALB/c mouse cellular DNA. BALB/c cell DNA was prepared as described in the text and sheared to 4 to 6S size. Hybridizations were carried out with 1,500 cpm of cDNA at 70°C in 0.75 M NaCl at 8 to 9 mg of cell DNA/ml with a 1.5 × 10⁷-nucleotide excess of cell DNA to cDNA. Aliquots of the hybridization mixture were removed and tested for both reannealed cell DNA and hybridized cDNA as described in the text. Hybridization curves are shown of BALB/c DNA reacting (●) MSV-MuLV common cDNA, (▲) MSV-specific cDNA, (○) and mouse globin cDNA; (□) cell DNA reannealing is shown also. The numbers represent actual hybridization values.*

quences as seen in Table 2 and Fig. 2c. Cat cells infected with M-MSV hybridized maximally (with 67% of the common cDNA), whereas all normal mouse cells tested showed only 50 to 60% reassociation of the probe with cell DNA relative to the cat cell hybridization values. The T_m of hybrids of "MSV-MuLV common" DNA and normal mouse cell DNA were reduced by 3 to 6° when compared with MSV or MuLV producing cells. However, already *Mus caroli* DNA had very little of the common nucleotide sequences, and the T_m was lower by 21°C indicating a significant lack of relationship. Rat cell DNA had about as little in the way of common nucleotide sequences as *M. caroli* DNA. However, the hamster DNA, although phylogenetically further removed from mice and rats, had a significantly greater percentage and a higher T_m of the common nucleotide sequences. Feline DNA also had a detectable presence of common nucleotide sequences of M-MSV, whereas canine cells had none. When melting profiles of mouse DNA unique sequences hybridized to DNA of different species were compared under the same conditions (Fig. 2d), the expected phylogenetic differences were confirmed (16). Accordingly, outside the mouse species the presence of the common M-MSV sequences was highly variable and quite unpredictable based on the expected evolutionary relationships.

Comparisons of evolutionary rates of some

TABLE 2. *Presence of M-MSV "common" sequences compared with the relationship of mouse unique sequences in cellular DNAs*

Species[a]	%[a] Hybridized	T_m[b] (°C)	ΔT_m (°C)	Copy no.	Unique sequences ΔT_m (°C)
MSV-MuLV-infected mouse cell	100	81	0	11	
MuLV-infected mouse cells	100	81	0	6	
MSV-infected cat cell[c]	100	78	3	6	
BALB/c mouse	60	75	6	7	0
NIH Swiss mouse	55	75	6	6	0
Mus caroli	6	60	21		5[d]
Fischer rat	8	60	21		15[e]
Hamster	20	68	13		18[e]
Cat	12	66	15		
Dog	2	60	21		
Chicken	3	60	21		
0					

[a] The same conditions used as in Table 1. The maximal actual hybridization value was 67% with a 3% background. The percentages of hybridization are normalized to 100% for the MSV-MuLV-infected cell DNA.

[b] T_m is calculated the same as in Table 1 except for *Mus caroli*, Fischer rat, dog, chicken, and 0 DNA in which case the T_m was taken as the temperature at which half of the bond cDNA at 50°C−0.12 M PB was eluted. This was done because these hybrids had no structured components to the melting profile above 60°C.

[c] MSV-infected cat cell is infected with m_1MSV. The MSV-MuLV common cDNA is from m_3MSV.

[d] Personal communication of R. Benveniste.

[e] Data of Laird et al. (16).

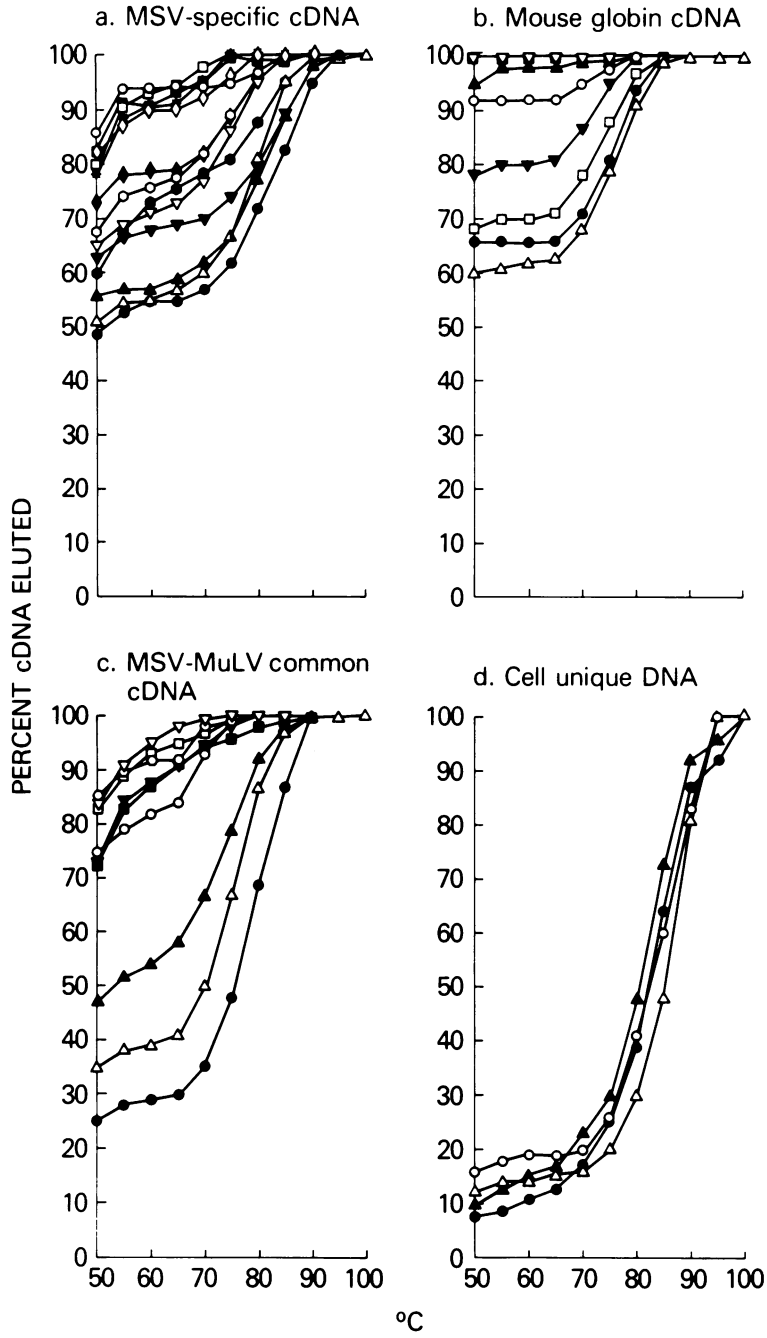

FIG. 2. *Thermal denaturation profiles on hydroxylapatite of cDNA cell DNA hybrids or reannealed cellular DNA. Denaturation profiles and controls were performed as described in the text after reaching a* C_0t *of 8,000 to 10,000 mol · s/liter. The hybrids used included (a) MSV-specific cDNA hybridized to cellular DNA of* (○) *rabbit,* (□) *calf,* (△) *Fischer rat,* (▽) *cotton rat,* (●) *BALB/c mouse,* (▲) *C3H mouse,* (▼) *C57BL mouse,* (⬡) *Mastomys,* (⬢) *Mus caroli,* (◇) *cat,* (◆) *hamster,* (✱) *dog; (b) mouse globin cDNA hybridized to cell DNA of* (■) *cat,* (▲) *dog,* (○) *hamster,* (●) *NIH Swiss mouse,* (▽) *chicken,* (△) *BALB/c mouse,* (□) *Mus caroli,* (▼) *Fischer rat; (c) MSV-MuLV common cDNA hybridized to cell DNA of* (●) *P521 (cat cell infected with MSV and FeLV),* (○) *hamster,* (△) *NIH Swiss mouse,* (▲) *BALB/c mouse,* (■) *Mus caroli,* (□) *chicken,* (▽) *dog,* (⬭) *cat,* (▼) *Fischer rat; (d) cellular DNA after reannealing of* (○) *cat,* (△) *human,* (▲) *BALB/c mouse,* (●) *NIH mouse.*

viral and cellular DNA sequences. Of the available norms describing phylogenetic sequence relationship of cell DNAs, the differences in the T_m of unique sequences has been used with the understanding that it is descriptive of an average value comprising many genes (16). However, it was clear from various sources that within a single species different genes evolve at different rates so that some genes, i.e., fibrinopeptides, are poorly conserved and others, such as cytochrome *c* and ribosomal RNA, are almost completely conserved in related species (7, 20). A single gene sequence such as hemoglobin whose product, function, and amino acid sequences are well known would be a useful standard of comparison for the rate of divergence of "MSV-specific" and "MSV-MuLV common" sequences. Table 3 and Fig. 2b present the rate of fixation of nucleotide substitutions for the globin gene as measured by the presence of homologous nucleotide sequences in the DNA of various species, and the reduction of T_m values. Globin DNA is apparently well conserved among species, and although the percentage of hybridization tended to diminish fairly rapidly, the T_m of the conserved regions is quite high. Carnivore globin DNA is much less related, and the avian globin is only minimally related. It is clear that *M. musculus* is closely related to *M. caroli*, less to rat, and still less to hamster. Thus, the probable taxonomic schema, unique sequence differences, and the changes within the hemoglobin gene are in reasonable agreement. The direct comparison of cDNA representing MSV-specific nucleotide sequences with globin cDNA and assorted cellular DNAs leads to the conclusion that MSV-specific copy was well conserved and evolved at approximately the rate of the globin gene. In contrast, common nucleotide sequences of M-MSV were much less well conserved and, compared with the globin DNA relationship, appear to be present or absent in a phylogenetically less predictable pattern.

TABLE 3. *Presence of mouse globin sequences in cellular DNAs*

Species	%	T_m[a] (°C)	ΔT_m (°C)	Evolutionary[b] distance (myr)
BALB/c mouse	100[c]	76	0	0
NIH Swiss mouse	92	77	0	0
Mus caroli	69	74	3	2
Fischer rat	57	72	5	5
Hamster	33	72	5	12
Rabbit	20	63	13	60
Cat	18	52	22	>60
Dog	29	54	24	
Chicken	8	50	26	
0	0	50	26	

[a] Without internal ^{32}P. Standard run in parallel on one of four hydroxylapatite water-jacketed columns. Cell DNA T_m (84 ± 1°C) tested on each run.

[b] Based on Simpson (25). *Mus caroli* divergence is estimated from unique sequence ΔT_m – see Table 2. The hybridizations were performed in the same tubes with "MSV-MuLV common" cDNA.

[c] Maximal actual hybridization value was 51% to BALB/c DNA with 0% background. All percentage hybridization values have been normalized to 100% for the homologous BALB/c DNA value.

DISCUSSION

Two subsets of representative cDNA were obtained from M-MSV: the MSV-specific portion, which was complementary to a quarter of the nucleotide sequences found only in M-MSV, and the "common" portion, representing the remainder of the M-MSV genome (13). The common nucleotide sequences are shared by M-MSV and MuLV; the Moloney strain of MuLV was the closest related, and the xenotropic murine oncornavirus from NZB mice was least related. The MSV-specific cDNA had no sequences in common with any known MuLV, xenotropic murine oncornavirus, Kirsten MSV, Harvey MSV, or mouse mammary tumor virus (12). The genetic content of the MSV-specific sequences is under investigation. The common sequences are definitely MuLV genes, as seen by the translation in nonmurine M-MSV-transformed cells of a p60 protein containing MuLV gs-1 antigenic determinants (19).

The availability of cDNA complementary to distinct portions of the M-MSV genome allowed the examination of their presence in normal mouse DNA, as well as in the DNA of related species. At least the majority of MSV-specific sequences were present as a single closely matched copy in the DNA of all normal mouse cells. In related *Mus* species the proportion of hybridizing sequences and the T_m of hybrids was diminished. Other rodents contained still less of a copy with more mismatching of sequences. Some rodents such as squirrels and guinea pigs had very little relationship to MSV-specific nucleotide sequences, which supports Simpson's model of extensive radiation among rodents and lack of common ancestors of *Sciuridae, Cavidae,* and *Muridae* families (25). The DNA of several other orders of mammals had only minimal sequences in common with MSV-specific cDNA. Accordingly, the evolution of MSV-specific sequences seemed to parallel normal speciation. Although only the majority (71 to 79%) of MSV-specific nucleotide sequences appear homologous to normal cell DNA, this lower final hybridization may be related to the

low copy number in these normal cells, as opposed to the MSV-producing control cells, which have 5 to 10 copies of MSV DNA per cell. Reconstruction experiments using MSV-infected cat cell DNA diluted with normal cat cell DNA suggest that the lower final hybridization of low copy number DNA is a hybridization artifact (11). The present findings are in agreement with the sarcoma-specific cDNA of ASV, a copy of which was found in normal chicken DNA and a related copy in each of several related avian species (27). There were also fewer of the ASV_{src} sequences in the related avian species. A point of difference lies in the fact that the M-MSV-specific sequences have a closely matched copy in normal mouse DNA, whereas the copy of ASV-specific sequences hybridizing with chicken DNA has a T_m reduced by 4°C.

In contrast, the common nucleotide sequences found in M-MSV were present in normal mouse DNA only to a lesser extent and already with significant mismatching. In *M. caroli* and the rat, the common sequences were essentially absent, but these partially recurred in the hamster and the cat. It is clear that common nucleotide sequences of M-MSV did not track with the expected evolutionary patterns. A similar result was previously obtained in extensive studies of primate oncornavirus evolution using cDNA probes derived from whole endogenous viruses. These "virogenes" radiated quite rapidly among species so that when examined by endogenous oncovirus cDNA, clear differences could be seen among primate species whose unique DNA sequences were otherwise very similar (3). In the avian oncovirus system, several studies indicated analogous lower degrees of relationship or absence of viral genes among closely related species (15, 17, 24, 27, 30). In the MuLV system as well, using cDNA probes from AKR type of MuLV, it was apparent that a set of virus-specific information was present in some strains of mice but absent in others (6). There is evidence that virogenes could at times be horizontally transmitted to unrelated species and from then on become a part of normal cellular DNA of that species (3). The present findings of more common sequences in the hamster and the cat than in *M. caroli* does in part follow from the known genetic composition of their oncornaviruses and their respective interspecies antigenic determinants (21).

The conservation of the MSV-specific nucleotide sequences among species was compared with one known mouse cellular gene, the hemoglobin gene, whose amino acid sequence, structure, and function are well known. It appeared that the rate of evolution of the globin gene was roughly comparable to that of the MSV-specific sequences, and that both sequences evolved more slowly than the average of cell DNA genes, as measured by unique-sequence hybridization. However, it is clear that within an organism, genes evolve at different rates, so that some genes such as those for fibrinopeptides are less well conserved whereas those coding for globin, insulin, or cytochromes are much better conserved (16). Two predictions arise from these findings: if the conservation of genes correlates with the requirement for functional integrity of the coded protein, which is important for survival of the animal, then the product of the M-MSV-specific sequences may serve some important role in mammalian development or survival. Second, because there is a greater conservation of amino acid primary sequences in the face of a potentially greater substitution in the coding of the bases, we might expect that the MSV-specific protein is more highly conserved and, once isolated or characterized, it could be readily traced throughout mammalia.

Finally, the finding of two subsets of distinct nucleotide sequences, covalently linked in the virus but evolving at totally different rates, is interesting. Perhaps, a ready explanation can be found in the previously detailed hypothesis, which states that oncogenic RNA viruses may arise by a recombination of endogenous oncornavirus sequences and some cellular control information, which is then transduced to susceptible cells (9, 23, 27). Similar hypotheses have also been advanced (28).

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LITERATURE CITED

1. **Bassin, R. H., N. Tuttle, and P. J. Fischinger.** 1970. Isolation of murine sarcoma virus transformed mouse cells which are negative for leukemia virus from agar suspension culture. Int. J. Cancer 6:95–107.
2. **Benveniste, R. E., and G. J. Todaro.** 1974a. Evolution of type C viral genes. I. Nucleic acid from baboon type C virus as a measure of divergence among primate species. Proc. Natl. Acad. Sci. U.S.A. 71:4513–4518.
3. **Benveniste, R. E., and G. J. Todaro.** 1974b. Evolution of C-type viral genes: inheritance of exogenously acquired viral genes. Nature (London) 252:456–459.
4. **Bernardi, G.** 1971. Chromatography of nucleic acids are hydroxylapatite columns, p. 95–147. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology. Academic Press Inc., New York.
5. **Britten, R. J., and D. E. Kohne.** 1968. Repeated sequences in DNA. Science 161:529–540.
6. **Chattopadhyay, S. K., D. R. Lowy, N. M. Teich, A. S.**

Levine, and W. P. Rowe. 1974. Qualitative and quantitative studies of AKR-type murine leukemia virus sequences in mouse DNA. Cold Spring Harbor Symp. Quant. Biol. 39:1085-1101.
7. Doolittle, R. F., and B. Blomback. 1964. Amino-acid sequence investigations of fibrinopeptides from various mammals: evolutionary implications. Nature (London) 202:147-152.
8. Fanshier, L., A. C. Garapin, T. McDonnell, A. Faras, W. Levinson, and J. Bishop. 1971. Deoxyribonucleic acid polymerase associated with avian tumor viruses: secondary structure of the deoxyribonucleic acid product. J. Virol. 7:77-86.
9. Fischinger, P. J., and D. K. Haapala. 1974. Oncoduction. A unifying hypothesis of viral carcinogenesis. Immunol. Cancer Prog. Exp. Tumor Res. 19:1-22.
10. Fischinger, P. J., C. O. Moore, and T. E. O'Connor. 1969. Isolation and identification of a helper virus found in the Moloney sarcoma-leukemia virus complex. J. Natl. Cancer Inst. 42:605-622.
11. Frankel, A. E., D. K. Haapala, R. L. Neubauer, and P. J. Fischinger. 1976. Elimination of the sarcoma genome from murine sarcoma virus transformed cat cells. Science 191:1264-1266.
12. Frankel, A. E., and P. J. Fischinger. 1976. Nucleotide sequences in mouse DNA and RNA specific for Moloney sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. 73:3705-3709.
13. Frankel, A. E., R. L. Neubauer, and P. J. Fischinger. 1976. Fractionation of DNA nucleotide transcripts from Moloney sarcoma virus and isolation of sarcoma virus-specific complementary DNA. J. Virol. 18:481-490.
14. Huebner, R. J., and G. J. Todaro. 1969. Oncogenes of RNA tumor viruses as determinants of cancer. Proc. Natl. Acad. Sci. U.S.A. 64:1087-1094.
15. Kang, C. Y., and H. M. Temin. 1974. Reticuloendotheliosis virus nucleic acid sequences in cellular DNA. J. Virol. 14:1179-1188.
16. Laird, C. D., B. L. McConaughy, and B. J. McCarthy. 1969. Rate of fixation of nucleotide substitutions in evolution. Nature (London) 224:149-154.
17. Neiman, P. E. 1973. Measurement of endogenous leukosis virus nucleotide sequences in the DNA of normal avian embryos by RNA-DNA hybridization. Virology 53:196-204.
18. Neiman, P. E., S. E. Wright, C. McMillin, and D. MacDonnell. 1974. Nucleotide sequence relationships of avian RNA tumor viruses: measurement of the deletion in a transformation-defective mutant of Rous sarcoma virus. J. Virol. 13:837-846.
19. Oskarsson, M. K., W. G. Robey, C. L. Harris, P. J. Fischinger, D. K. Haapala, and G. F. Vande Woude. 1975. A p60 polypeptide in the feline leukemia virus pseudotype of Moloney sarcoma virus with murine leukemia virus p30 antigenic determinants. Proc. Natl. Acad. Sci. U.S.A. 72:2380-2384.
20. Pinder, J. C., H. J. Gould, and I. Smith. 1969. Conservation of the structure of ribosomal RNA during evolution. J. Mol. Biol. 40:289-298.
21. Schäfer, W., H. Bauer, D. P. Bolognesi, P. Fischinger, H. Frank, H. Gelderblom, J. Lange, and M. V. Nermut. 1974. Studies on structural antigenic properties of C-type viruses, p. 115-140. *In* Molecular studies in viral neoplasia. The Williams & Wilkins Co., Baltimore, Md.
22. Scolnick, E. M., R. J. Goldberg, and W. P. Parks. 1974. A biochemical and genetic analysis of mammalian RNA-containing sarcoma viruses. Cold Spring Harbor Symp. Quant. Biol. 39:885-895.
23. Scolnick, E. M., R. J. Goldberg, and D. Williams. 1976. Characterization of rat genetic sequences of Kirsten sarcoma virus: distinct class of endogenous rat type C viral sequences. J. Virol. 18:559-566.
24. Soyab, M., and M. A. Baluda. 1975. Homology between avian oncornavirus RNAs and DNA from several avian species. J. Virol. 16:1492-1502.
25. Simpson, G. G. 1959. The nature and origin of supraspecific taxa. Cold Spring Harbor Symp. Quant. Biol. 24:255-271.
26. Stehelin, D., R. V. Guntaka, H. E. Varmus, and J. M. Bishop. 1976. Purification of DNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. J. Mol. Biol. 101:349-365.
27. Stehelin, D., H. E. Varmus, J. M. Bishop, and P. K. Vogt. 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature (London) 260:170-173.
28. Temin, H. M. 1974. On the origin of the genes for neoplasia: G. H. A. Clowes Memorial Lecture. Cancer Res. 34:2835-2841.
29. Thomas, C. A., V. I. Bens, and T. J. Kelley. 1966. Isolation of high molecular weight DNA from bacteria and cell nuclei, p. 535-552. *In* G. C. Cantoni and D. R. Davies (ed.). Harper & Row, Publishers, New York.
30. Varmus, H. E., S. Heasley, and J. M. Bishop. 1974. Use of DNA-DNA annealing to detect new virus-specific DNA sequences in chicken embryo fibroblasts after infection by avian sarcoma virus. J. Virol. 14:895-903.